CLAIMS

What is claimed is:

1. A method for selecting a single chain antibody (scFv) against a peptide target in a yeast, comprising:

expressing a library of scFv fusion proteins in yeast cells, each scFv fusion protein comprising either an activation domain or a DNA binding domain of a transcription activator and a scFv, the scFv comprising a V_H of antibody whose sequence varies within the library, a V_L of antibody whose sequence varies within the library independently of the V_H, and a linker peptide which links the V_H and V_L;

expressing a target fusion protein in the yeast cells expressing the scFv fusion proteins, the target fusion protein comprising either the DNA binding domain or the activation domain of the transcription activator which is not comprised in the scFv fusion proteins, and a target peptide; and

selecting those yeast cells in which a reporter gene is expressed, the expression of the reporter gene being activated by a reconstituted transcriptional activator formed by binding of the scFv fusion protein to the target fusion protein.

2. The method of claim 1, wherein expressing the library of scFv fusion proteins includes transforming a library of scFv expression vectors into the yeast cells which contain a reporter construct comprising the reporter gene whose expression is under transcriptional control of the reconstituted transcription activator, each scFv expression vector comprising

a first transcription sequence encoding either the activation domain or the DNA binding domain of the transcription activator, and

a scFv sequence encoding one of the scFv antibodies.

3. The method of claim 2, wherein expressing a target fusion protein includes transforming a target expression vector into the yeast cells simultaneously or sequentially with the library of scFv expression vectors, the target expression vector comprising

a second transcription sequence encoding either the activation domain or the DNA binding domain of the transcription activator which is not expressed by the library of scFv expression vectors; and

a target sequence encoding the target peptide; and

expressing the target fusion protein from the target expression vector.

4. The method of claim 1, wherein the steps of expressing the library of scFv fusion proteins and expressing the target fusion protein include causing mating between first and second populations of haploid yeast cells of opposite mating types,

wherein

the first population of haploid yeast cells comprises

a library of scFv expression vectors for the library of scFv fusion proteins, each scFv expression vector comprising

a first transcription sequence encoding either the activation domain or the DNA binding domain of the transcription activator, and

a scFv sequence encoding one of the scFv antibodies;

the second population of haploid yeast cells comprises a target expression vector comprising

a second transcription sequence encoding either the activation domain or the DNA binding domain of the transcription activator which is not expressed by the library of tester expression vectors, and

a target sequence encoding the target peptide; and

either the first or second population of haploid yeast cells comprises a reporter construct comprising the reporter gene whose expression is under transcriptional control of the transcription activator.

5. The method of claim 4, wherein the haploid yeast cells of opposite mating types are α and a type strains of yeast.

6. The method of claim 5, wherein the mating between the first and second

populations of haploid yeast cells of α and a type strains is in a rich nutritional culture medium.

7. The method of claim 1, wherein the diversity of scFv antibodies in the library of scFv fusion proteins is at least $1x10^4$.

8. The method of claim 1, wherein the diversity of scFv antibodies in the library of scFv fusion proteins is at least $1x10^6$.

9. The method of claim 1, wherein the diversity of scFv antibodies in the library of scFv fusion proteins is at least $1x10^7$.

10. The method of claim 1, wherein the target peptide has a length of 5-100 aa.

11. The method of claim 1, wherein the target peptide has a length of 10-80 aa.

12. The method of claim 1, wherein the target peptide has a length of 20-60 aa.

13. The method of claim 1, wherein the target peptide comprises a peptide fragment of a membrane protein.

14. The method of claim 13, wherein the peptide fragment of the membrane protein is an extracellular domain of the membrane protein.

15. The method of claim 13, wherein the membrane protein is selected from the group consisting of receptors for growth factors, insulin receptor, MHC proteins, CD3 receptor, T cell receptors, cytokine receptors, tyrosine-kinase-associated receptors and G-protein coupled receptors.

16. The method of claim 15, wherein receptors for growth factors are selected from the group consisting of receptors for vascular endothelial growth factor, epidermal growth factor, transforming growth factor, fibroblast growth factor, platelet derived growth factor, and insulin-like growth factor.

17. The method of claim 15, wherein the MHC protein is class I or class II MHC protein.

18. The method of claim 15, wherein the cytokine receptor is selected from interleukin-1 receptor, interleukin-2 receptor, interleukin-8 receptor, and interleukin-12 receptor,

19. The method of claim 15, wherein the tyrosine-kinase-associated receptors is selected from the group consisting of *Src, Yes, Fgr, Flt, Lck, Lyn, Hck,* and *Blk.*

20. The method of claim 15, wherein the G-protein coupled receptor is a coreceptors for HIV.

21. The method of claim 20, wherein the coreceptor for HIV is selected from the group consisting of CXCR4, CCR5, CCR1, CCR2b, CCR3, CCR4, CCR8, CXCR1, CXCR2, CXCR3, CX_3CR1, STRL33/BONZO and GPR15/BOB.

22. The method of claim 1, wherein the V_H and V_L are encoded by variable regions of immunoglobulin genes of a human, non-human primate, or rodent.

23. The method of claim 1, wherein the V_H and V_L are encoded respectively by a heavy-chain variable region and a light-chain variable region of a human immunoglobulin gene.

24. The method of claim 1, wherein the V_H is encoded by a heavy-chain variable region of a first human immunoglobulin gene, and the V_L is encoded by a light chain variable region of a second human immunoglobulin gene different from the first human immunoglobulin gene.

25. The method of claim 1, wherein the transcription activator is selected from the group consisting of GAL4, GCN4, and ADR1 transcription activators.

26. The method of claim 1, wherein the protein encoded by the reporter gene is selected from the group consisting of β-galactosidase, α-galactosidase, luciferase, β-glucuronidase, chloramphenicol acetyl transferase, secreted embryonic alkaline phosphatase, green fluorescent protein, enhanced blue fluorescent protein, enhanced yellow fluorescent protein, and enhanced cyan fluorescent protein.

27. The method of claim 2 or 3, further comprising:

isolating the scFv expression vector from the selected yeast cells; and

mutagenizing the V_H and V_L in the isolated scFv expression vectors to form a library of mutagenized expression vectors.

28. The method of claim 27, wherein the mutagenesis is selected from the group consisting of error-prone PCR mutagenesis, site-directed mutagenesis, DNA shuffling and combinations thereof.

29. The method of claim 27, further comprising:

transforming the library of mutagenized expression vectors into the yeast cells,

transforming the target expression vector into the yeast cells simultaneously or sequentially with the library of mutagenized expression vectors;

expressing the target fusion protein from the target expression vector; and

selecting those yeast cells in which the reporter gene is expressed, the expression of the reporter gene being activated by binding of the tester fusion protein to the target fusion protein.